Ann. Occup. Hyg., Vol. 56, No. 7, pp. 815–828, 2012

doi:10.1093/annhyg/mes011

A Detailed Urinary Excretion Time Course Study of Captan and Folpet Biomarkers in Workers for the Estimation of Dose, Main Route-of-Entry and Most Appropriate Sampling and Analysis Strategies

AURÉLIE BERTHET[1,2], ROBERTO HEREDIA-ORTIZ[1], DAVID VERNEZ[2], BRIGITTA DANUSER[2] and MICHÈLE BOUCHARD[1]*

[1]Department of Environmental and Occupational Health, School of Public Health, Université de Montréal, PO Box 6128, Main Station, Montreal, Quebec, Canada H3C 3J7; [2]Institute for Work and Health, University of Lausanne and University of Geneva, Bugnon 21, 1011 Lausanne, Switzerland

Received 13 July 2011; in final form 27 January 2012; published online 16 March 2012

Captan and folpet are two fungicides largely used in agriculture, but biomonitoring data are mostly limited to measurements of captan metabolite concentrations in spot urine samples of workers, which complicate interpretation of results in terms of internal dose estimation, daily variations according to tasks performed, and most plausible routes of exposure. This study aimed at performing repeated biological measurements of exposure to captan and folpet in field workers (i) to better assess internal dose along with main routes-of-entry according to tasks and (ii) to establish most appropriate sampling and analysis strategies. The detailed urinary excretion time courses of specific and non-specific biomarkers of exposure to captan and folpet were established in tree farmers ($n = 2$) and grape growers ($n = 3$) over a typical workweek (seven consecutive days), including spraying and harvest activities. The impact of the expression of urinary measurements [excretion rate values adjusted or not for creatinine or cumulative amounts over given time periods (8, 12, and 24 h)] was evaluated. Absorbed doses and main routes-of-entry were then estimated from the 24-h cumulative urinary amounts through the use of a kinetic model. The time courses showed that exposure levels were higher during spraying than harvest activities. Model simulations also suggest a limited absorption in the studied workers and an exposure mostly through the dermal route. It further pointed out the advantage of expressing biomarker values in terms of body weight-adjusted amounts in repeated 24-h urine collections as compared to concentrations or excretion rates in spot samples, without the necessity for creatinine corrections.

Keywords: biomonitoring; captan; dose reconstruction spraying activities; exposure assessment; folpet; field workers; re-entry activities

INTRODUCTION

Captan (*N*-(trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide) and folpet (*N*-[trichloromethylthio]phthalimide) are the two common dicarboximide fungicides used in various crops. Captan was patented by Kittleson (1952) and first introduced in 1951, while folpet was first registered as a pesticide in 1948. Both compounds have thus been used by workers for almost 60 years, but their health effects are still controversial and mostly documented from animal toxicity studies.

The United States Environmental Protection Agency (US EPA) (1975; 1999) initially classified both fungicides as probable human carcinogens

*Author to whom correspondence should be addressed.
Tel: +1-514-343-6111 ext 1640; fax: +1-514-343-2200;
e-mail: michele.bouchard@umontreal.ca

(B2) based on an increased incidence of duodenum tumors in mice chronically exposed to high doses by gavage. However, in 2004, the agency revised the classification of captan and changed it to 'not likely' considering that the doses administered to the mice were much higher than those encountered in occupational settings and induced proliferation of nascent tumors through cytotoxicity and cell hyperplasia (US EPA, 2004; Gordon, 2007). Similarly, Cohen *et al.* (2010) demonstrated in their review that folpet is not likely to be a human carcinogen for the same reasons as captan, and Greenburg *et al.* (2008) found no evidence of an increase in the incidence of cancer among applicators exposed to captan over a 9-year period. Captan has also been classified as a Group 3 carcinogen (or limited evidence of carcinogenicity in experimental animals) by the International Agency for Research on Cancer (1987) and in Group A3 (or confirmed animal carcinogen with unknown relevance to humans) by the American Conference of Governmental Industrial Hygienists (ACGIH, 2010). On the other hand, folpet is not listed in the index of the latter two organizations.

Even though no systemic toxicity of captan and folpet was reported in humans, both fungicides are considered as sensitizers and strong irritants of the eyes, skin, and respiratory airways (Hayes, 1982; ACGIH, 1991; Edwards *et al.*, 1991; Trochimowicz *et al.*, 1991; Tomlin, 1997; US EPA, 1999; US EPA, 2004; NIOSH, 2007; Costa, 2008; Gordon, 2010). A few studies reported skin problems (i.e. allergic reactions and dermatitis) in workers exposed to captan or folpet (Burroughs and Hora, 1982; Lisi *et al.*, 1987; Guo *et al.*, 1996). Burroughs and Hora (1982) also mentioned that 48.4% of workers employed in a fungicide production plant stated having eye problems (i.e. burning, itching, and tearing of eyes) and 58.1% declared suffering from respiratory problems (i.e. dry throat, sore throat, coughing, wheezing, shortness of breath, and difficulty breathing) ($n = 66$). As a result, occupational guidelines were proposed for captan, namely a Threshold Value Limit-Time Weighted Average (TVL-TWA) of 5 mg m^{-3} (ACGIH, 2010) or a recommended exposure limit (REL®) of 5 mg m^{-3} (NIOSH, 2007), but none are available to date for folpet, except the recommendation by the US EPA (1999) to wear gloves when handling the product. Therefore, risks related to occupational exposure to captan and especially folpet are not well defined.

Worker exposure and absorption may also be affected by multiple factors and conditions. For instance, in addition to frequent reported factors, such as the dose, exposure duration, vehicle, skin conditions and its composition, or physicochemical characteristics of compounds, others factors such as the type of crop, meteorological conditions, the delay of re-entry, or work habits and practices may also be important determinants of exposure and absorption (Zweig *et al.*, 1985; Winterlin *et al.*, 1986; Tielemans *et al.*, 1999; Stewart *et al.*, 2001; Geer *et al.*, 2004; Hughes *et al.*, 2006).

To identify factors or activities most likely to increase worker exposure to captan, some authors have performed environmental measurements using personal dosimeters or skin pads, while assessing the impact of wearing masks or hand washing (Oudbier *et al.*, 1974; Stevens and Davis, 1981; Burroughs and Hora, 1982; Mcjilton *et al.*, 1983; Zweig *et al.*, 1985; Ritcey *et al.*, 1987; Tielemans *et al.*, 1999). However, external exposure measurements are known to present limitations and to lead to overestimations of true absorbed doses. The best means of accurately assessing worker exposure to such type of compound is recognized to be through biological monitoring since it allows estimating actual rather than potential absorption by workers and integrating exposure by all routes (He, 1993; Woollen, 1993; de Cock *et al.*, 1995).

Some field studies have attempted to associate environmental measurements with biomonitoring data to assess captan exposure (Hansen *et al.*, 1978; Winterlin *et al.*, 1984; Winterlin *et al.*, 1986; Maddy *et al.*, 1989; Lavy *et al.*, 1993; de Cock *et al.*, 1995; Krieger and Dinoff, 2000; Hines *et al.*, 2008), but poor correlations were obtained. These studies, as well as those of van Welie *et al.* (1991) and of Verberk *et al.* (1990) which used only biomonitoring, assessed worker exposure for a maximum three consecutive days, with incomplete collections; typical tasks involving potential exposure to captan (e.g. spraying and harvest activities) were also assessed in workers. However, according to some authors (Woollen, 1993; Thongsinthusak *et al.*, 1999; Ross *et al.*, 2001), to accurately estimate absorption, especially through the dermal route, the optimal sampling protocol would be to collect 24-h voids for 7 days in a worker performing different tasks during a workweek and thus subjected to various exposure scenarios.

By comparison with captan, there is a paucity of data on occupational exposure to folpet, although it is also widely used in agriculture. The only available data come from a health hazard report conducted by the National Institute for Occupational Safety and Health (NIOSH) (Burroughs and Hora, 1982) to evaluate captan and folpet exposure in ~60 employees working in a fungicide production plant through environmental and medical evaluation.

There is thus a need to better assess occupational exposure to these two fungicides and this can effectively be achieved through biomonitoring. Nonetheless, such approach requires a minimum knowledge of the toxicokinetics of the compound under study, hence of major metabolites, together with a sensitive analytical method for their quantification in accessible biological matrices (Wester and Maibach, 1983; Woollen, 1993). For the biomonitoring of worker exposure to captan, tetrahydrophthalimide (THPI) was quantified in the published studies as a urinary metabolite of captan due to its stability. Its interest as a biomarker of exposure was confirmed by our previous kinetic studies in volunteers orally and dermally exposed to captan in controlled conditions (Berthet *et al.*, 2011b,d). According to the time course data of Berthet *et al.* (2011b,d), phthalimide (PI) and total ring-metabolites of folpet [expressed as phthalic acid equivalents (PA_{eq})] also proved to be two potentially useful biomarkers of folpet exposure.

This study thus aimed at (i) better assessing occupational exposure to captan and folpet, through repeated biological measurements in field workers ($n = 5$) following spraying and harvest activities (internal dose and main route-of-exposure) and use of toxicokinetic modeling as well as (ii) establishing most appropriate sampling and analysis strategies.

MATERIALS AND METHODS

Study design

The detailed time profiles of key biomarkers of exposure to captan and folpet were characterized in the urine of agricultural workers subjected to different exposure scenarios, preparing/mixing/loading/spraying activities, and harvest activities following the 48 h required delay of re-entry. Captan and folpet ring-metabolites were quantified in pre-seasonal urines and, for each exposure scenario, in all urines voided over seven consecutive days. From these data, the dose absorbed by workers and main route-of-entry were estimated using toxicokinetic models previously developed by our team, which allow to reconstruct absorbed doses of captan and folpet from biomarker data considering different exposure scenarios (Heredia-Ortiz and Bouchard, 2011; Heredia-Ortiz *et al.*, 2011).

The experimental protocol and consent forms were approved by the Permanent Ethics Committee for Clinical Research of the Faculty of Biology and Medicine of the University of Lausanne (protocol 134/07) and the Research Ethics Committee of the Faculty of Medicine of the University of Montreal (CERFM (06)#227). All the participants gave their written consent and were informed of the risks of participating and their right to withdraw from the study at anytime.

Studied workers

Participants were recruited on a voluntary basis among tree farmers and grape growers living within 100 km from Lausanne (Switzerland). Approximately 12 workers were contacted, but only 5 persons accepted to participate, namely 2 tree farmers (exposed to captan) and 3 grape growers (exposed to folpet), due to the restrictive protocol. All participants were male workers aged between 35 and 55 years old, weighing 74–115 kg, and measuring 178–192 cm. They were healthy and non-smokers and underwent a medical examination by an occupational physician prior to enrollment.

Urine sample collection

Urine sample collections were conducted over seven consecutive days (or 168 h) following two different types of exposure, namely spraying activities (including preparing, mixing, and loading tasks) and harvest activities following the required re-entry delay (i.e. pruning and thinning), except for one grape grower performing harvest activities who collected all his urine voided over a 72-h period. During the collection period, several spraying techniques were used by the studied workers (i.e. tractors with closed or half-opened cabins, small airblast sprayers, and back air sprayers) and sampling was conducted during the season period presumed to be associated with worst exposure scenarios.

More specifically, to determine urinary baseline levels of the studied metabolites, a pre-seasonal complete first-morning void was collected for each worker; during this period, they were not occupationally exposed to captan or folpet. At the beginning of the fungicide treatment period, workers were then asked to provide all urine voided during the course of a typical workweek involving a spraying episode of captan or folpet (in general, a 168-h collection period with spraying the first sampling day). Each void was collected in a separate polypropylene Nalgene® bottles of 1 l; workers were asked to indicate the date and time of urine collection on the pre-coded bottle labels.

During the high season of thinning activities and pruning of vineyards or orchards, the same workers were again asked to provide a second round of urine collection. During this period, the vegetation was dense and abundant, and workers were easily in contact with treated leaves. All urine voided during the

course of a typical workweek involving harvest activities were thus collected following the required delay of re-entry (in general, a 168-h collection period with harvest activities on several days). At least 2 weeks separated the two exposure scenarios.

Once collected, urine samples were kept in the refrigerator and daily picked up by our team. Total urine volume per void was then measured upon arrival at the laboratory. To allow repeated analysis while avoiding possible degradation due to freezing and thawing of samples, each urine collection was then aliquoted in four labeled tubes of 15 ml and one bottle of 120 ml prior to storage at −20°C until analysis.

In addition, during each urinary collection period, workers were invited to complete a timesheet with the actual time of each voiding and to indicate whether or not there were any urine losses. They were also asked to fill a questionnaire to document personal factors (weight and height), information related to spraying and harvest activities (i.e. commercial product name, application days, techniques, and tasks), work habits (i.e. safety equipments, decontamination tasks, and hand washing), treatments (i.e. other captan/folpet treatments or other pesticides sprayed during the study period), life habits (i.e. physical activities and smoking), medication intake (including ibuprofen), and possible symptoms during workdays. Distinct questionnaires were elaborated for the two exposure scenarios and adapted to the tasks performed.

Sample analysis

THPI and PI. THPI and PI were quantified in urine according to the method of Berthet *et al.* (2011c). In short, THPI and PI were isolated by solid phase extraction, eluted in dichloromethane, and analyzed by liquid chromatography—atmospheric pressure chemical ionization–tandem mass spectrometry (LC/APCI-MS/MS) in negative ion mode. The fragments analyzed were m/z 149.4/95.6 for THPI, m/z 156.1/95.6 for the internal standard THPI-d, and m/z 145.8 for PI (no fragmentation). The analytical limit of detection in urine was 3.8 and 7.7 nmol l^{-1} for THPI and PI, respectively. The quantification of THPI or PI was obtained from standard calibration curves prepared in urine or plasma adjusted by the THPI-d internal standard peak area.

Phthalic acid equivalents. Total ring-metabolites of folpet, expressed as PA_{eq}, were measured according to the method of Berthet *et al.* (2011a). Briefly, urine samples were subjected to an acid hydrolysis prior to liquid–liquid extraction with ethyl acetate and derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide. Analysis was then performed by gas chromatography–mass spectrometry (GC–MS). The ions monitored were trimethylsilyl (TMS) phthalic acid with m/z 295 and the internal standard TMS methylhippuric acid with m/z 220. The quantification was obtained from standard calibration curves of phthalic acid prepared in urine and adjusted by the methylhippuric acid internal standard peak height. The analytical limit of detection was 60.2 nmol l^{-1} urine.

Creatinine. Creatinine was measured in urine by an alkaline picric acid method with deproteinization, namely by the Jaffé method with deproteinization (enzymatic colorimetric test PAP from Boehringer, Mannheim, Germany).

To adjust THPI, PI, and PA_{eq} urinary excretion rates by creatinine contents, the following equation, described by Viau *et al.* (2004), was used:

$$\left[\left(\frac{\Delta\text{metabolite}}{\Delta t}\right)\right]_{\text{Adj}} = \left[\left(\frac{\Delta\text{metabolite}}{\Delta t}\right)\right]_i \times \frac{\left[\frac{\Delta\text{Creatinine}}{\Delta t}\right]_{\text{mean}}}{\left[\frac{\Delta\text{Creatinine}}{\Delta t}\right]_i},$$

where $\left[\left(\frac{\Delta\text{metabolite}}{\Delta t}\right)\right]_{\text{Adj}}$ is the adjusted excretion rate of the studied metabolite, $\left[\left(\frac{\Delta\text{metabolite}}{\Delta t}\right)\right]_i$ is the excretion rate observed over a determined time interval i, $\left[\frac{\Delta\text{Creatinine}}{\Delta t}\right]_{\text{mean}}$ is the average creatinine excretion rate for the total study period, and $\left[\frac{\Delta\text{Creatinine}}{\Delta t}\right]_i$ is the average creatinine excretion rate over a determined time interval i.

Toxicokinetic modeling

Multi-compartment toxicokinetic models were developed to describe the time courses of captan and folpet key biomarkers in accessible biological matrices following multi-routes of exposure (Heredia-Ortiz and Bouchard, 2011; Heredia-Ortiz *et al.*, 2011). A specific model was built to describe the kinetics of THPI metabolite of captan. On the other hand, the kinetics of PI and PA_{eq} metabolites of folpet were modeled separately. These models were used in the current study to reconstruct the absorbed doses of these fungicides in workers from serial urinary biomarker measurements and to obtain an indication of the predominant route of exposure for these workers.

Briefly, in the models, the body was represented by compartments. The rates of change in the amounts of compounds or its metabolites in the different compartments were represented by a set of linear first-order ordinary differential equations. Kinetics of fungicides and their experimentally relevant metabolites were modeled for three different exposure routes: oral, dermal, and inhalation. To describe oral exposure, the considered compartments were the parent compound and its almost instantaneously generated metabolites in the gastrointestinal tract, the

body burden of metabolites in blood and in tissues in dynamical equilibrium with blood, both monitored and non-monitored, and the different excretion compartments representing cumulative amounts of monitored metabolites in urine and feces. To simulate dermal exposure, the epidermis and dermis were represented by distinct compartments (except for the kinetics of PA_{eq} given the absence of measured blood time course of PA_{eq}, which simplifies model representation). Finally, inhalation exposure was modeled with direct inputs to the blood compartment due to the rapid absorption of both fungicides through the respiratory tract (Canal-Raffin *et al.*, 2006, 2007). All amounts in models were initially expressed on a molar basis (see supplementary information, *Annals of Occupational Hygiene* online, for model representation and parameter values).

RESULTS

Worker exposure

Table 1 summarizes the characteristics, exposure conditions, and activities of the workers under study. In the case of workers exposed to captan, field spraying was conducted using tractors with a cabin; they did not wear masks or coveralls during spraying or harvest activities, and only one wore gloves during

Table 1. Characteristics of captan or folpet exposure for each worker following fungicide treatment and harvest activities.

	Captan exposure[a]		Folpet exposure[b]		
	Worker #1	Worker #2	Worker #1	Worker #2	Worker #3
Application activities[c]					
Active ingredient, %	80% captan	80% captan	50% folpet	80% folpet	25% folpet
Amounts (kg)	4 kg (or 1 kg ha^{-1})	NR	1 kg ha^{-1}	3.5 kg (or 1 kg ha^{-1})	1 kg ha^{-1}
Water volume	400 l ha^{-1}	500 l ha^{-1}	NR	700 l (200 l ha^{-1})	NR
Treated area (ha)	2.5	5	NR	3.3	NR
Spraying date	07–08/05/2009	08/08/2009	25/06/2009	07–08/05/2009	16/05/2009
Total spraying duration, h	2	8	4	6	3
Spraying technique	Airblast pulled by a tractor with an opened cabin	Airblast pulled by a tractor with a closed cabin	Airblast sprayer	Airblast sprayer	Airblast sprayer and a back air spray
Safety equipment worn during application	Leather shoes	Rubber boots	Full-face helmet with filter	Complete forced air helmet	Half-face helmet with filter
			Coveralls	Tissue coveralls	Tissue hat
	Waterproof gloves	Cap	Waterproof gloves	Safety shoes	Waterproof gloves
Symptoms	No	No	Eye irritation	No	Eye irritation
Harvest activities					
Active ingredient, %	80% captan	80% captan	50% folpet	80% folpet	60% folpet
Harvesting date	15/06/2009	17–20/06/2009	25/06/2009	27–30/05/2009	22–26/06/2009
Total harvesting duration, h	5	30	6	36	45
Symptoms	No	No	No	No	Eye irritation

NR, not reported.

[a]Workers exposed to captan wore the same safety equipment during preparation and cleaning as described for application. No personal protective equipment was worn during harvesting period. Hands were washed after spraying and harvesting, and the clothes were removed at home at the end of the workday for both activities. For Worker 1, preparation was conducted inside and gloves only were decontaminated with water post-spraying. Conversely, for Worker 2, preparation was conducted outside and no decontamination of equipment was performed post-spraying.

[b]Workers exposed to folpet wore the same safety equipment during preparation and cleaning as described for application (excluding helmet), except Worker 1 who had no personal protective equipment during cleaning. Preparation was conducted outside for Worker 1 and inside with a ventilation system for the two other workers. Mask or helmet, gloves (Workers 1–3), tissue coveralls (Worker 2), and hat (Worker 3) were decontaminated with soap and water post-spraying. The three workers washed their hands after spraying and harvesting. Worker 1 removed his clothes at home at the end of the workday after spraying activities and at work after harvest activities, Worker 2 at work at the end of the workday after spraying activities and at home after harvest activities, and Worker 3 at home at the end of the workday for both activities. For harvesting, Worker 2 wore gloves and pants, and Worker 3 wore rubber gloves only.

[c]All workers performed mixing, loading, and material cleaning activities in addition to spaying.

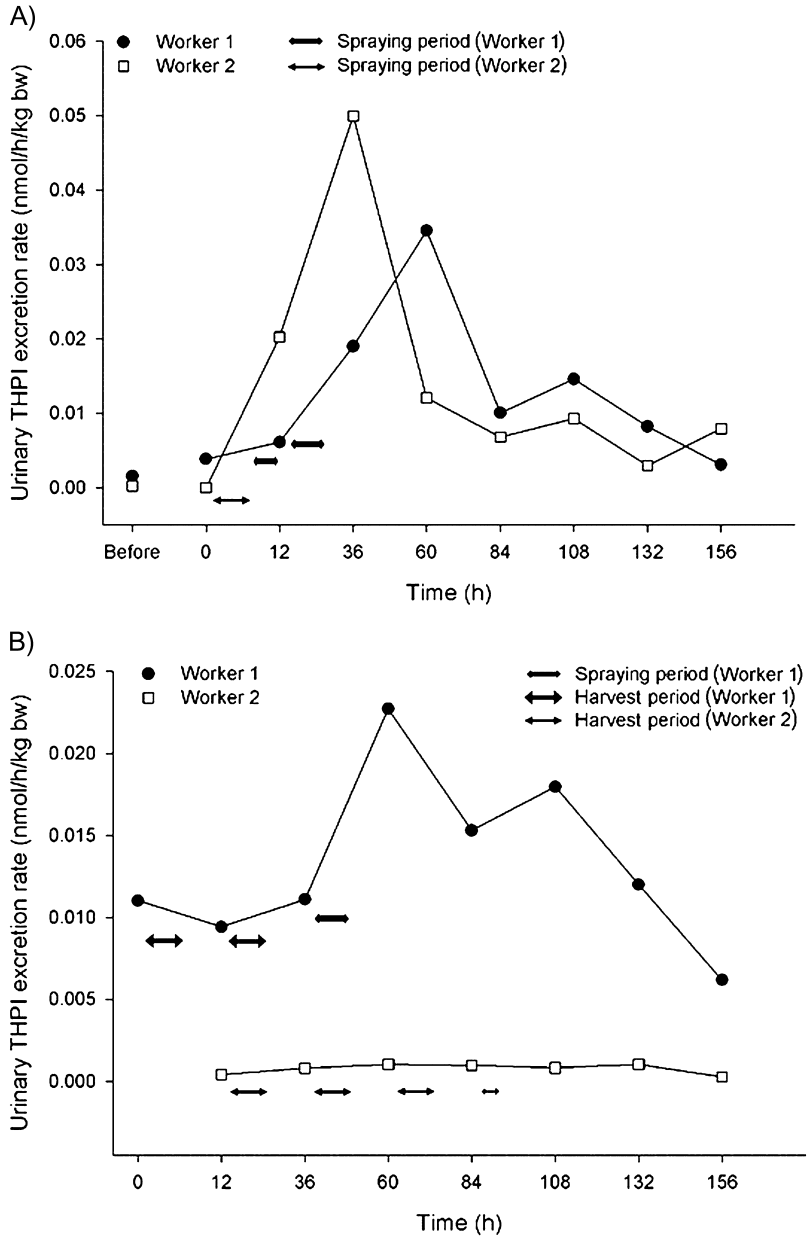

Fig. 1. Time courses of THPI urinary excretion rate (expressed as nanomoles per hour per kilogram of body weight) over a 168-h period in two workers exposed to captan following spraying activities (A) and harvest activities in a captan-treated area (B). Arrows represent treatment period or harvesting period (for the two workers).

preparation, mixing, loading, and cleaning tasks but not during harvest activities. In the case of workers exposed to folpet, they tended to protect themselves better since all wore masks during preparation and spraying activities as well as gloves and pants during harvest activities. This increased protection was probably due to the fact that they used airblast sprayers or back air sprayers to apply folpet and were thus more likely to be in contact with the applied fungicide. Two workers reported eye irritations following folpet spraying and one of these two workers also reported eye irritation following harvest activities, while no symptoms were mentioned by workers exposed to captan.

To assess the importance of exposure due to spraying and harvest activities, considering the previously

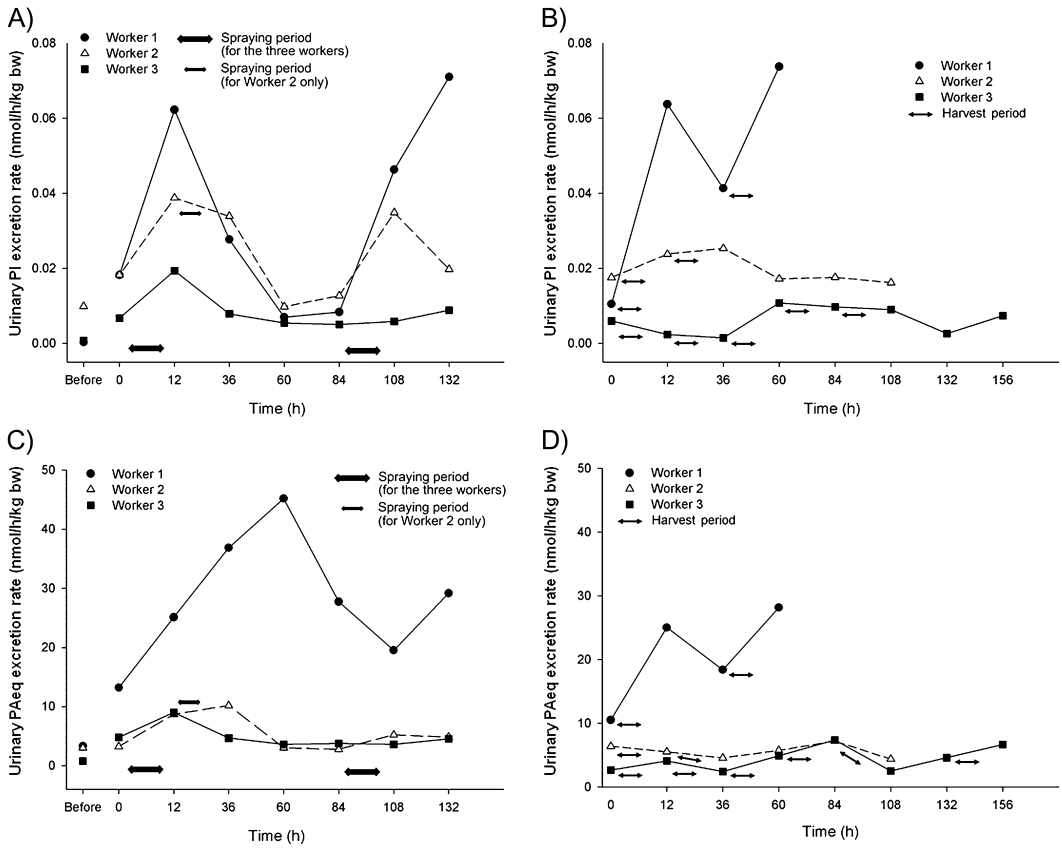

Fig. 2. Time courses of PI (A and B) and PA_{eq} (C and D) urinary excretion rate (expressed as nanomoles per hour per kilogram of body weight) over a 168-h period in three workers exposed to folpet following spraying activities (A and C) and harvest activities after a delay-of-rentry (B and D). Arrows represent treatment period or harvesting period (for the three workers).

mentioned exposure conditions, THPI was measured in urine as a biomarker of exposure to captan, while PI and PA_{eq} were quantified to assess folpet exposure. Figure 1 depicts the urinary time profiles of THPI in the two studied workers over a 7-day period following captan spraying or harvesting in a captan-treated area; Fig. 2 presents corresponding time profiles for PI and PA_{eq} biomarkers of exposure to folpet in the three studied workers. Exposure to captan was found to be higher during spraying than harvest activities. This is particularly apparent for Worker 2 since he was barely exposed during harvest activities, with values close to pre-seasonal levels. Worker 2 appeared more exposed than Worker 1 over the spraying period, but he did not wear gloves during work and he manipulated larger amounts of captan given that he had to treat a broader area. Similarly, workers seemed more exposed to folpet during spraying than harvest activities. This is especially apparent from the time profiles of PI and PA_{eq} in Worker 2. However, differences in excretion values between both activities were less noticeable than for captan. As presented in Table 1, workers exposed to folpet were better protected during spraying activities contrary to workers exposed to captan.

From Fig. 2, the urinary time courses of PI and PA_{eq} in workers following spraying and harvesting can also be compared. As expected, similar profiles were obtained for both biomarkers, except for Worker 1 during spraying period. This allowed pointing out a substantial baseline level of phthalic acid in workers due to an exposure other than folpet.

Creatinine adjustments and timed collections

Figures 3 and 4 show the impact of creatinine adjustment on the urinary excretion time course of THPI and PI along with profile variations when expressing urinary results in terms of spot or pooled measurements over 8-, 12-, or 24-h periods. Results show that creatinine adjustment had little effect on the time courses of biomarkers in spot or pooled samples as non-adjusted and creatinine-adjusted rate

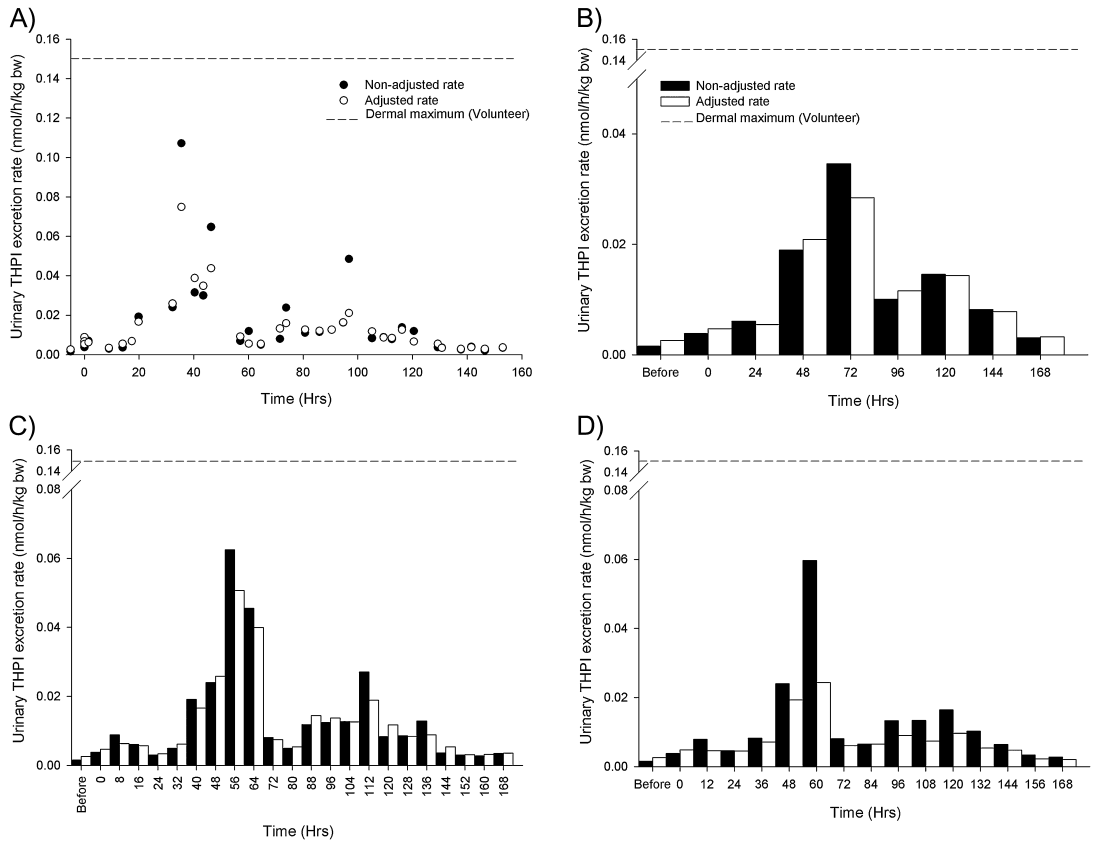

Fig. 3. Time courses of THPI excretion rates (expressed as nanomoles per hour per kilogram of body weight) non-adjusted (open symbols) and adjusted by creatinine (closed symbols) in spot urines (A) or 8-h (C), 12-h, (D) and 24-h (B) collections in a worker exposed to captan during spraying activities. The dermal maximum lines represent maximum values measured in the urine of volunteers exposed to 10 mg kg^{-1} of captan by the dermal route (Berthet *et al*., 2011b).

profiles were found to quantitatively evolve in a similar manner. This was even more evident with pooled urines, especially 24-h urine collections. Figures 3 and 4 also show that excretion rate profiles were less variable when urines were pooled over the longest period of time, hence 24 h. In contrast with 24-h collections, it was also less obvious to infer on the main route-of-exposure from individual voids due to the important variations between some data points.

Exposure route simulations

Figure 5 shows an example of model simulation of the time courses of THPI and PI metabolites in workers for both spraying and harvest scenarios, considering the various possible absorption routes (inhalation, dermal, or oral). Simulations of a dermal exposure scenario for both captan and folpet, during spraying period as well as harvest activities, provided the closest description of the observed time courses as compared to oral and inhalation scenarios. However, contrary to workers exposed to captan, it was less obvious from observed time courses of folpet metabolites that dermal absorption was in all cases the predominant exposure route of folpet exposure for workers.

DISCUSSION

Results of the present study show notable variations in captan and folpet biomarker levels according to field tasks, such as spraying or harvest activities. Through biomonitoring, it was thus evidenced that workers were more exposed during application than harvest activities. Model simulations of urinary time course data considering various exposure route scenarios further indicated that captan was mainly absorbed through the skin following both spraying and harvesting. For folpet, main exposure route is

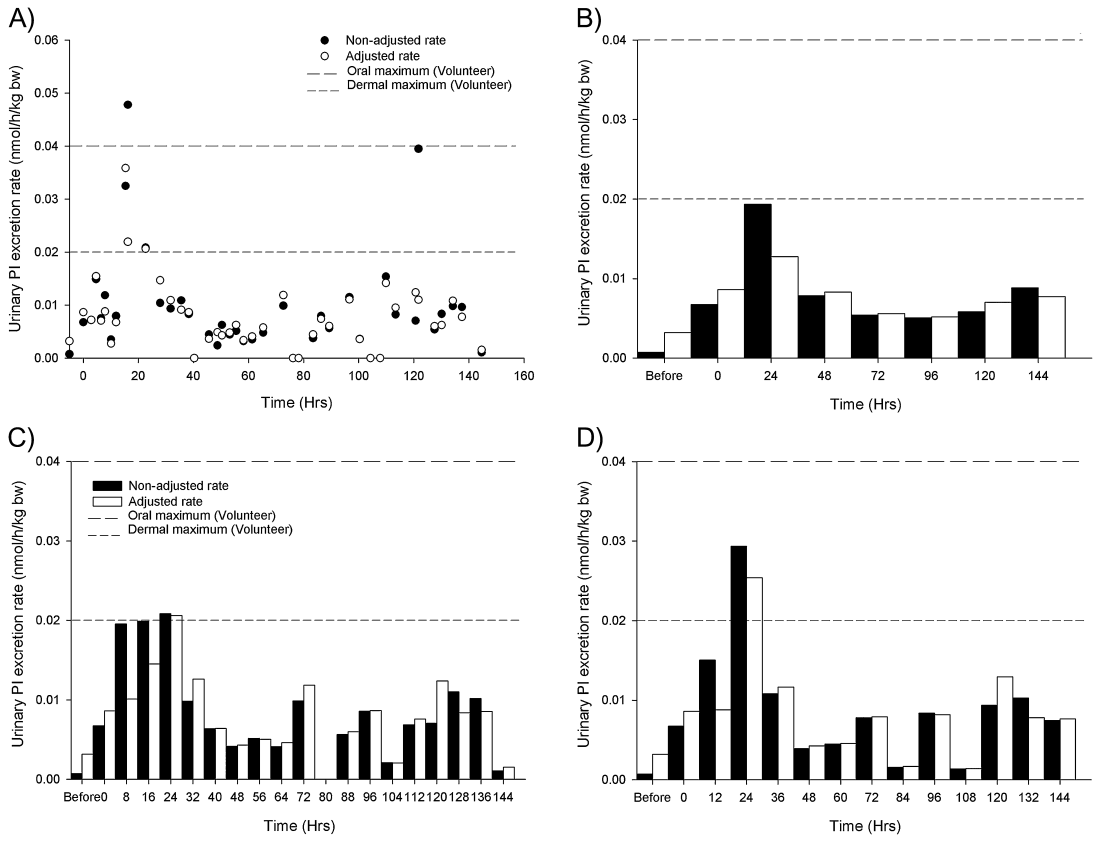

Fig. 4. Time courses of PI excretion rates (expressed as nanomoles per hour per kilogram of body weight) non-adjusted (open symbols) and adjusted by creatinine (closed symbols) in spot urines (A) or 8-h (C), 12-h, (D) and 24-h (B) collections in a worker exposed to folpet during spraying activities. The dermal and oral maximum lines represent maximum values measured in the urine of volunteers exposed to 10 mg kg^{-1} of folpet by the dermal route or 1 mg kg^{-1} by the oral route (Berthet *et al*., 2011a,b).

less obvious but model simulations of a dermal scenario provided the closest approximation of the observed data (Fig. 5). This does not exclude a certain concomitant inhalation exposure in the studied workers. In addition, collections of complete voids over seven consecutive days allowed confirming that measurements of biomarkers in 24-h collections without creatinine normalization provided the most reliable assessment of worker exposure to captan and folpet.

Comparison of exposure levels between spraying and harvest activities

According to biomonitoring results in the studied workers (Figs. 1 and 2), exposure to captan and folpet was more important during spraying period than harvest activities. This is probably due mostly to mixing and loading prior to spaying as suggested by de Cock *et al.* (1998a). However, exposure during spraying was of shorter duration and less frequent, and workers were more protected (Table 1). The same observations were reported in studies assessing captan exposure through spot measurements (Winterlin *et al.*, 1986; Krieger, 1995; de Cock *et al.*, 1998b; Tielemans *et al.*, 1999; Geer *et al.*, 2004).

Thus, harvest activities following a re-entry in treated fields resulted in limited dermal absorption according to biomonitoring results in the studied workers (Figs. 1 and 2), even though half-life of captan on leaf surfaces was estimated to be between 2.5 and 24 days (Frank *et al.*, 1983; Winterlin *et al.*, 1984; Stamper *et al.*, 1987; el-Zemaity, 1988; Alary *et al.*, 1995; Cabras *et al.*, 1997, 2000; de Cock *et al.*, 1998b; Tielemans *et al.*, 1999; Phalen and Que Hee, 2003; US EPA, 2004). For folpet, however, results of the current study do not exclude the possibility that workers could be also exposed by oral or inhalation routes.

Although workers of the current study were more exposed during spraying than harvesting, urinary

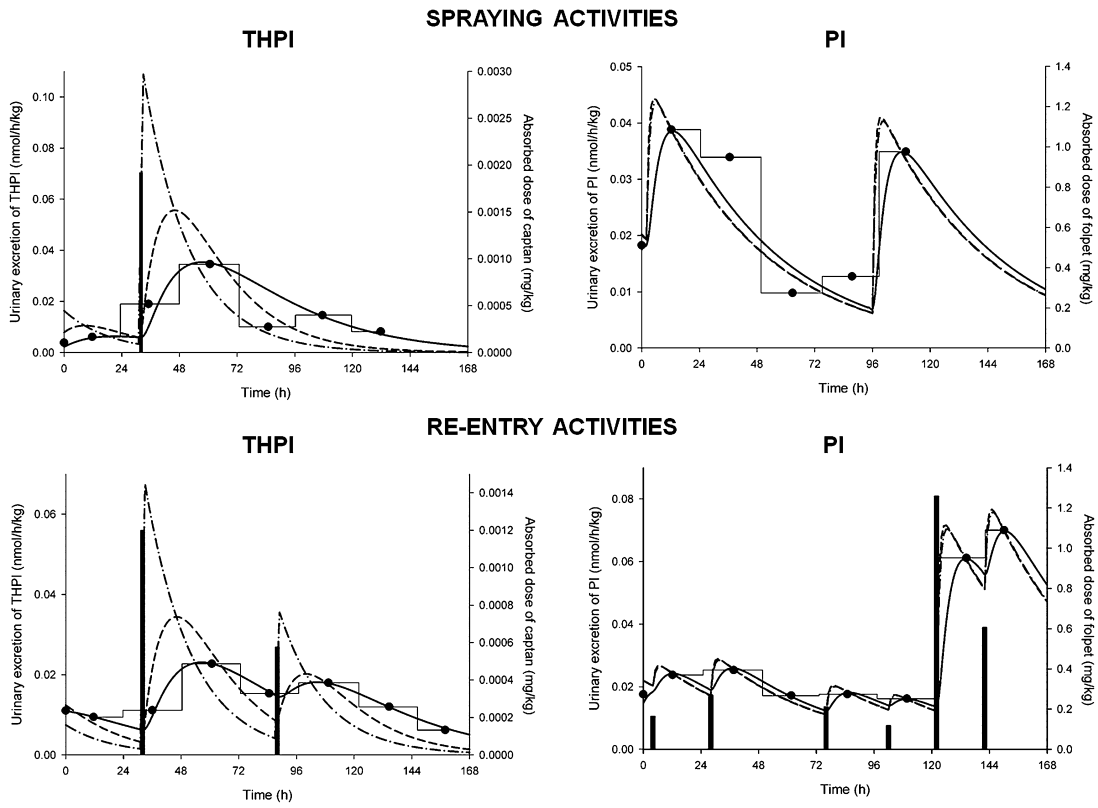

Fig. 5. Dermal model simulations (solid line) compared with experimental data on the time courses of THPI and PI in the urine of a worker exposed to captan or folpet over a workweek following spraying and harvest activity periods. Solid circles and gray outlines show experimental rate values in 24-h collections, black bars represent the corresponding simulated absorbed dose scenario (at given time points) with values on the right axis, and lines characterize model simulations for a dermal route exposure (solid lines), an oral route exposure (dashed lines), and an inhalation exposure (dotted–dashed lines).

excretion values of THPI were in general lower than those reported in the literature and summarized in Table 2. For applicators exposed to captan, our mean 24-h excretion values following the beginning of treatment period were equivalent to those obtained by Hines *et al.* (2008), but lower than most of the other available studies. Likewise, our workers exposed to captan during harvesting exhibited lower THPI concentrations than those of other published studies (Winterlin *et al.*, 1984; Winterlin *et al.*, 1986; Krieger and Dinoff, 2000). In the other studies, larger amounts of captan were however applied and a wider treatment area was covered compared to the current study.

In addition, urinary THPI concentrations in workers exposed to captan were lower than maximum values previously observed in a controlled kinetic time course study in volunteers dermally applied 10 mg kg^{-1} of captan on 80 cm^2 of the forearm during 24 h (Berthet *et al.*, 2011d) (maximum concentration obtained for both workers of 44.9 nmol l^{-1} compared to average maximum concentration for volunteers of 180 nmol l^{-1}). On the other hand, urinary PI concentrations in two of the three studied folpet workers (Workers 2 and 3) reached values similar to those observed in a kinetic time course study in volunteers dermally exposed to folpet (i.e. maximum of 18.3 nmol l^{-1} in workers compared to 17.7 nmol l^{-1} in volunteers), while maximum values in the third worker (Worker 1) were rather higher (i.e. maximum of 26.9 nmol l^{-1}).

As for the major route of exposure, current results (see model simulations in Fig. 5) are in line with those previously reported and showing that dermal absorption is the primary route-of-entry for both mixers/loaders/applicators and re-entry workers in contact with pesticides (Gunther *et al.*, 1977; Ritcey *et al.*, 1987; Thongsinthusak *et al.*, 1999; Ross *et al.*, 2001; Geer *et al.*, 2004). In particular, de Cock *et al.* (1995) and Hansen *et al.* (1978) found that

Table 2. Published mean concentrations of THPI in the urine of workers exposed to captan following different activities in various types of crops.

References	Mean concentration/amount of THPI[a]			N[b]	Worker exposure scenario[c]				
	Pre-shift urine	Post-shift urine	24-h urine		Activity[d]	Crops[e]	Duration[f]	Total treated area (mean)[g]	Captan amount[h]
Winterlin *et al.* (1984)									
	NE	<30 μg l^{-1}	NE	1	Applicator/ loader/mixer	Strawberries	8 h	0.5–2 acres	2.2 lbs of AI $acre^{-1}$
	NE	58–60 μg l^{-1}	NE	12	Harvesters	Strawberries	8 h	0.5–2 acres	
Winterlin *et al.* (1986)									
	50 μg l^{-1}	63 μg l^{-1}	57 μg l^{-1}	3	Loader/ mixer/applicators	Grapes	8 h	36–40 acres	2.0–2.5 lbs AI
	32 μg l^{-1}	50 μg l^{-1}	47 μg l^{-1}	16	Harvesters	Grapes	8 h	37 acres	2.0 lbs AI
Maddy *et al.* (1989)									
	NE	NE	5 μg l^{-1}	10	Pickers	Strawberries	3 days	72 acres	4 lbs AI $acre^{-1}$
Verberk *et al.* (1990)									
	8 μmol mol^{-1} creatinine	NE	20 μmol mol^{-1} creatinine	6	Dipping bulbs in captan solution	Flower bulbs	NE	NE	NE
van Welie *et al.* (1991)									
	NE	7.2 μg l^{-1} (5.4 μmol mol^{-1} creatinine)	NE	8	Applicators	Fruit	NE	NE	NE
Lavy *et al.* (1993)									
	No THPI founded			73	Applicators, weeders, scouts, or packers	Conifer seedling	12 consecutive weeks	NE	1.4 or 54.4 kg
de Cock et al. (1995)									
	NE	NE	11.5 μg	14	Applicators	Fruit	93 min	14.8 acres	10.4 kg
Krieger and Dinoff (2000)									
	No background	NE	2.0–5.3 μg day^{-1}	41	Harvesters	Strawberries	3 days	102–162 acres	3.75 lbs $acre^{-1}$
Hines *et al.* (2008)									
	NE	4.05 μg l^{-1}	3.55 μg l^{-1}	14	Applicators	Strawberries	211 min	5.3 acres	7.9 kg

Table 2. *Continued*

References	Mean concentration/amount of THPI[a]			N^b	Worker exposure scenario[c]				
	Pre-shift urine	Post-shift urine	24-h urine		Activity[d]	Crops[e]	Duration[f]	Total treated area (mean)[g]	Captan amount[h]
Our study									
	0.26 µg l^{-1i} (1.69 nmol l^{-1})	2.70 µg l^{-1i} (17.8 nmol l^{-1})	2.95 µg l^{-1i} (19.5 nmol l^{-1})	2	Loader/ mixer/applicators	Apple trees	2–8 h	2.5–5 acres	1 kg AI $acre^{-1}$
	0.14 µg l^{-1i} (0.93 nmol l^{-1})	0.89 µg l^{-1i} (5.88 nmol l^{-1})	0.59 µg l^{-1i} (3.90 nmol l^{-1})	2	Pruning and thinning	Apple trees	5–30 h	2.5–5 acres	1 kg AI $acre^{-1}$

NE, not estimated; AI, active ingredient; lbs, pounds.
[a]Mean concentration or amounts of THPI measured in urine of workers exposed to captan.
[b]Number of workers participating in the study.
[c]Information concerning worker exposure to captan: activities during exposure, type of studied crops, duration of exposure, mean total treated area, and amounts of captan applied on fields during the studied period.
[d]Activities performed by workers during the studied period of exposure to captan.
[e]Studied crop fields.
[f]Duration of worker exposure to captan during the study period.
[g]Mean total area (expressed in acres) treated with captan.
[h]Mean amounts of captan (as active ingredient) sprayed during the study period.
[i]Mean THPI levels calculated from data of both workers.

respiratory exposure route to captan was minor compared to dermal absorption.

Parameters influencing exposure assessment through biomonitoring

Biomonitoring in field workers allows estimating doses truly absorbed in workers whatever the exposure scenario (Woollen, 1993). However, depending on tasks and activities, workers are not exposed constantly or equally during a workday or a week. When feasible, it is thus preferable to obtain complete daily collections over several days to assess most accurately worker exposure, as suggested by some authors (Woollen, 1993; Thongsinthusak *et al.*, 1999; Ross *et al.*, 2001), instead of spot urine samples. This was also particularly evident from our results, showing that an overestimation or underestimation of exposure may be induced with punctual urines, as illustrated in Figs. 3 and 4, since there are significant void-to-void variations in metabolite concentrations and urinary volumes (Woollen, 1993; Spencer *et al.*, 1995). With combined 8-h urine collections, time profiles were better defined for the studied metabolites than with spot samples; however, it was the daily (24-h) variations in biomarker levels which allowed to reproduce most closely the time course in workers using the toxicokinetic models previously developed (Heredia-Ortiz and Bouchard, 2011; Heredia-Ortiz *et al.*, 2011).

Creatinine normalization of metabolite excretion rates, as proposed by Viau *et al.* (2004), also appeared unnecessary in this study since adjusted values were close to non-adjusted values, especially in 24-h urine collections (Figs. 3 and 4). Consequently, when feasible, using complete 24-h voids over a week, including days off, appears to be the most reliable procedure to estimate worker exposure to captan and particularly folpet, given the paucity of available biomonitoring data.

Nonetheless, complete 24-h urine collections may be burdensome for exposed workers and hardly feasible in routine biological monitoring. Based on current results and those of a previous study in volunteers dermally exposed to captan and folpet (Berthet *et al.*, 2011d), one alternative is to collect first morning void, end-of-shift sample, and last evening void during three consecutive days. Spot samples collected on several days are still needed to properly assess exposure as peak levels of THPI and PI in blood were reached only 24 and 10 h after a dermal exposure to captan and folpet in volunteers, respectively, with ensuing mean elimination half-lives ($t^1/_2$) of 24.7 and 29.7 h, respectively (Berthet *et al.*, 2011d). An approach more amenable to routine

monitoring would be to collect complete first morning voids in workers the day following the onset of an exposure period and to record urine collection time as well as last void time prior to collection. Using modeling and provided there is some information available on the exposure scenario and urine collection time with reference to the exposure period, it then becomes possible to infer on most plausible absorbed doses in the studied workers based on amounts of THPI or PI in this timed collection.

In summary, the present biomonitoring study used detailed repeated measurements along with kinetic modeling tools to better assess worker exposure to captan and folpet and main route-of-entry. Despite the limited number of participants, sufficient data were obtained to confirm results reported in the literature for captan and to provide new data on folpet exposure. However, more investigations are needed to further document exposure to folpet in workers and confirm main absorption route.

SUPPLEMENTARY DATA

Supplementary data can be found at http://annhyg.oxfordjournals.org/.

FUNDING

Agence Nationale de Sécurité Sanitaire de l'alimentation, de l'environnement et du Travail (ANSES). APR-2006-12-14 (EST 2006/1/3/DROZ)

Acknowledgements—Authors wish to thank the five workers to their participation and their attention to respect the protocol as well as Dr Michèle Berode, Gregory Plateel, and the technicians P. Stefan, M. Nobel, C. Kohler, and P. Storti (Institute for Work and Health) for their help in analytical analysis. We also thank Dr Khanh Huynh (Institute for Work and Health) for his help with the LC/MS/APCI system. Aurélie Berthet also received a scholarship from the Institut de recherche Robert-Sauvé en santé et sécurité du travail du Québec (IRSST).

REFERENCES

ACGIH. (1991) Docuentation of the threshold limit values and biological exposure indices. Vols. 1–3, 6th edn. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH. (2010) TLVs® and BEIs®. Based on documentation of the threshold limit values for chemical substances and physical agents and biological exposure indices. 7th edn. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Alary J, Bescos D, Monge MC *et al*. (1995) Laboratory simulation of captan residues degradation during apple processing. Food Chem; 54: 205–11.

Berthet A, Berode M, Bouchard M. (2011a) Gas-chromatography mass-spectrometry determination of phtalic acid in human urineas a biomarker of folpet exposure. Anal Bioanal Chem; 400: 493–502.

Berthet A, Bouchard M, Danuser B. (2011b) Toxicokinetics of captan and folpet biomarkers in orally exposed volunteers. J Appl Toxicol; 32: 194–201.

Berthet A, Bouchard M, Schüpfer P *et al*. (2011c) A liquid chromatography-tandem mass spectrometry (LC/APCI-MS/MS) method for the quantification of captan and folpet phthalimide metabolites in human plasma and urine. Anal Bioanal Chem; 399: 2243–5.

Berthet A, Bouchard M, Vernez D. (2011d) Toxicokinetics of captan and folpet biomarkers in dermally exposed volunteers. J Appl Toxicol; 32: 202–9.

Burroughs GE, Hora J. (1982) HETA-80-147-1076. Health hazard evaluation. Cincinnati, OH: National Institute for Occupational Safety and Health.

Cabras P, Angioni A, Caboni P *et al*. (2000) Distribution of folpet on the grape surface after treatment. J Agric Food Chem; 48: 915–16.

Cabras P, Angioni A, Garau VL *et al*. (1997) Persistence and metabolism of folpet in grapes and wine. J Agric Food Chem; 45: 476–9.

Canal-Raffin M, L'azou B, Martinez B *et al*. (2006) Impact of folpet on respiratory system: in vitro studies. Epidemiology; 17: S90.

Canal-Raffin M, L'azou B, Martinez B *et al*. (2007) Physicochemical characteristics and bronchial epithelial cell cytotoxicity of Folpan 80 WG(R) and Myco 500(R), two commercial forms of folpet. Part Fibre Toxicol; 4: 8.

Cohen SM, Gordon EB, Singh P *et al*. (2010) Carcinogenic mode of action of folpet in mice and evaluation of its relevance to humans. Crit Rev Toxicol; 40: 531–45.

Costa LG. (2008) Toxic effects of pesticides: the basic science of poisons. In Casarett LJ, Doull J and Klaassen CD, editors. Casarett and Doull's toxicology: the basic science of poisons. New York: McGraw-Hill; pp. 883–930. ISBN 978 0 07 147051 3.

de Cock J, Heederik D, Hoek F *et al*. (1995) Urinary excretion of tetrahydrophtalimide in fruit growers with dermal exposure to captan. Am J Ind Med; 28: 245–56.

de Cock J, Heederik D, Kromhout H *et al*. (1998a) Determinants of exposure to captan in fruit growing. Am Ind Hyg Assoc J; 59: 166–72.

de Cock J, Heederik D, Kromhout H *et al*. (1998b) Exposure to captan in fruit growing. Am Ind Hyg Assoc J; 59: 158–65.

Edwards R, Ferry DG, Templ WA. (1991) Fungicides and related compounds. In Hayes WJ and Laws ER, editors. Handbook of pesticide toxicology. San Diego, CA: Academic Press; pp. 1409–70. ISBN 978 0 12 334160 0.

el-Zemaity MS. (1988) Residues of captan and folpet on greenhouse tomatoes with emphasis on the effect of storing, washing, and cooking on their removal. Bull Environ Contam Toxicol; 40: 74–9.

Frank R, Braun HE, Stanek J. (1983) Removal of captan from treated apples. Arch Environ Contam Toxicol; 12: 265–69.

Geer LA, Cardello N, Dellarco MJ *et al*. (2004) Comparative analysis of passive dosimetry and biomonitoring for assessing chlorpyrifos exposure in pesticide workers. Ann Occup Hyg; 48: 683–95.

Gordon E. (2007) Captan: transition from 'B2' to 'not likely'. How pesticide registrants affected the EPA Cancer Classification Update. J Appl Toxicol; 27: 519–26.

Gordon EB. (2010) Captan and folpet. In Krieger RI, editor. Handbook of pesticide toxicology. New York: Elsevier; pp. 1915–49. ISBN 978 0 12 374367 1.

Greenburg DL, Rusiecki J, Koutros S *et al*. (2008) Cancer incidence among pesticide applicators exposed to captan in the Agricultural Health Study. Cancer Causes Control; 19: 1401–7.

Gunther FA, Iwata Y, Carman GE *et al*. (1977) The citrus reentry problem: research on its causes and effects, and approaches to its minimization. Residue Rev; 67: 1–132.

Guo YLL, Wang BJ, Lee CC *et al*. (1996) Prevalence of dermatoses and skin sensitisation associated with use of pesticides in fruit farmers of southern Taiwan. Occup Environ Med; 53: 427–31.

Hansen JD, Schneider BA, Olive BM *et al*. (1978) Personnel safety and foliage residue in an orchard spray program using azinphosmethyl and captan. Arch Environ Contam Toxicol; 7: 63–71.

Hayes WJ. (1982) Fungicides and related compounds. In Hayes WJ, editor. Pesticides studied in man. Baltimore, MD: Williams and Wilkins. pp. 578–622. ISBN 978 0 68 303896 5.

He F. (1993) Biological monitoring of occupational pesticides exposure. Int Arch Occup Environ Health; 65: S69–76.

Heredia-Ortiz R, Berthet A, Bouchard M. (2011) Toxicokinetic modeling of folpet fungicide and its ring-biomarkers of exposure in humans. J Appl Toxicol; doi:10.1002/jat.1782.

Heredia-Ortiz R, Bouchard M. (2011) Toxicokinetic modeling of captan fungicide and its tetrahydrophthalimide biomarker of exposure in humans. Toxicol Lett; doi:10.1016/j.toxlet.2011.09.023.

Hines CJ, Deddens JA, Jaycox LB *et al*. (2008) Captan exposure and evaluation of a pesticide exposure algorithm among orchard pesticide applicators in the agricultural health study. Ann Occup Hyg; 52: 153–66.

Hughes EA, Zalts A, Ojeda JJ *et al*. (2006) Analytical method for assessing potential dermal exposure to captan, using whole body dosimetry, in small vegetable production units in Argentina. Pest Manag Sci; 62: 811–18.

International Agency for Research on Cancer. (1987) Captan. In Overall evaluations of carcinogenicity: an updating of IARC Monographs. Volumes 1–42, [Supplement 7]. Lyon, France: World Health Organization.

Kittleson AR. (1952) A new class of organic fungicides. Science; 115: 84–6.

Krieger RI. (1995) Pesticide exposure assessment. Toxicol Lett; 82–83: 65–72.

Krieger RI, Dinoff TM. (2000) Captan fungicide exposures of strawberry harvesters using THPI as a urinary biomarker. Arch Environ Contam Toxicol; 38: 398–403.

Lavy TL, Mattice JD, Massey JH *et al*. (1993) Measurements of year-long exposure to tree nursery workers using multiple pesticides. Arch Environ Contam Toxicol; 24: 123–44.

Lisi P, Caraffini S, Assalve D. (1987) Irritation and sensitization potential of pesticides. Contact Dermatitis; 17: 212–18.

Maddy KT, Krieger RI, Oconnell L *et al*. (1989) Use of biological monitoring data from pesticide users in making pesticide regulatory decisions in California—study of captan exposure of strawberry pickers. Acs Symp Ser; 382: 338–53.

Mcjilton CE, Berckman GE, Deer HM. (1983) Captan exposure in apple orchards. Am Ind Hyg Assoc J; 44: 209–10.

NIOSH. (2007) NIOSH pocket guide to chemical hazards. Cincinnati, OH: National Institute for Occupational Safety and Health . DHHS Publication No 2005-149, -454: 2007.

Oudbier AJ, Bloomer AW, Price HA *et al*. (1974) Respiratory route of pesticide exposure as a potential health hazard. Bull Environ Contam Toxicol; 12: 1–9.

Phalen RN, Que Hee SS. (2003) Permeation of captan through disposable nitrile glove. J Hazard Mater; 100: 95–107.

Ritcey G, Frank R, McEwen FL *et al*. (1987) Captan residues on strawberries and estimates of exposure to pickers. Bull Environ Contam Toxicol; 38: 840–6.

Ross JH, Driver JH, Cochran RC *et al*. (2001) Could pesticide toxicology studies be more relevant to occupational risk assessment? Ann Occup Hyg; 45: S5–17.

Spencer JR, Sanborn JR, Hernandez BZ *et al*. (1995) Long vs. short monitoring intervals for peach harvesters exposed to foliar azinphos-methyl residues. Toxicol Lett; 78: 17–24.

Stamper JH, Nigg HN, Queen RM. (1987) Dislodgeable captan residues at Florida strawberry farms. Chemosphere; 16: 1257–71.

Stevens ER, Davis JE. (1981) Potential exposure of workers during seed potato treatment with captan. Bull Environ Contam Toxicol; 26: 681–8.

Stewart PA, Prince JK, Colt JS *et al*. (2001) A method for assessing occupational pesticide exposures of farmworkers. Am J Ind Med; 40: 561–70.

Thongsinthusak T, Ross JH, Saiz SG *et al*. (1999) Estimation of dermal absorption using the exponential saturation model. Regul Toxicol Pharmacol; 29: 37–43.

Tielemans E, Louwerse E, de Cock J *et al*. (1999) Exposure to fungicides in fruit growing: re entry time as a predictor for dermal exposure. Am Ind Hyg Assoc J; 60: 789–93.

Tomlin C. (1997) The pesticide manual—world compendium. 10th edn. Thornton Heath, UK: British Crop Protection Council. ISBN 978 1 90 139626 3.

Trochimowicz HJ, Kennedy GL, Krivanek ND. (1991) Heterocyclic and miscelaneous nitrogen compounds. In Clayton GD and Clayton FE, editors. Patty's industrial hygiene and toxicology. Vol. 2, part E, 4th edn. New York: Wiley; pp. 3285–521. ISBN 978 0 47 101282 5.

United States Environmental Protection Agency. (1975) EPA/540-1-75-012: 1975. Initial scientific and mini-economic review of captan. Washington, DC: United States Environmental Protection Agency, Office of Pesticide Programs, Criteria and Evaluation Division.

US EPA. (1999) EPA 738-R-99–011: 1999. Registration eligibility decision (RED)—folpet. Washington, DC: United States Environmental Protection Agency.

US EPA. (2004) 69 Fed. Reg. 68357-68360: 2004. Amendment to the 1999 captan Registration Eligibility Decision (RED). Captan: cancer reclassification. Washington, DC: United States Environmental Protection Agency.

van Welie RT, van DP, Lamme EK *et al*. (1991) Determination of tetrahydrophtalimide and 2-thiothiazolidine-4-carboxylic acid, urinary metabolites of the fungicide captan, in rats and humans. Int Arch Occup Environ Health; 63: 181–6.

Verberk MM, Brouwer DH, Brouwer EJ *et al*. (1990) Health effects of pesticides in the flower-bulb culture in Holland. Med Lav; 81: 530–41.

Viau C, Lafontaine M, Payan JP. (2004) Creatinine normalization in biological monitoring revisited: the case of 1-hydroxypyrene. Int Arch Occup Environ Health; 77: 177–85.

Wester RC, Maibach HI. (1983) Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. Drug Metab Rev; 14: 169–205.

Winterlin WL, Kilgore WW, Mourer CR *et al*. (1984) Worker reentry studies for captan applied to strawberries in California. J Agric Food Chem; 32: 664–72.

Winterlin WL, Kilgore WW, Mourer CR *et al*. (1986) Worker reentry into captan-treated grape fields in California. Arch Environ Contam Toxicol; 15: 301–11.

Woollen BH. (1993) Biological monitoring for pesticide absorption. Ann Occup Hyg; 37: 525–40.

Zweig G, Leffingwell JT, Popendorf W. (1985) The relationship between dermal pesticide exposure by fruit harvesters and dislodgeable foliar residues. J Environ Sci Health B; 20: 27–59.